

Table EI - Plasmids, strains, primers used in the study.

Plasmid	Description	Reference
pRMC2	Plasmid with $P_{xyl/tetO}$ promoter inducible by anhydrotetracycline. 100 $\mu\text{g/ml}$ Amp ^R in <i>Escherichia coli</i> , 10 $\mu\text{g/ml}$ Cam ^R .	[1]
pRMC2+MsrA	pRMC2 plasmid, bearing sequence coding for Msr(A) from <i>Staphylococcus haemolyticus</i> JCSC1435 chromosome (NC_007168.1) with additional C-terminal Gly-(His) ₆ tag and inserted into KpnI, SacI restriction sites of pRMC2 vector.	This study
Strains	Description	Reference
XL1-Blue	<i>Escherichia coli</i> cloning strain XL1-Blue	Stratagene
RN4220 + pRMC2	<i>Staphylococcus aureus</i> RN4220 strain transformed by pRMC2. 10 $\mu\text{g/ml}$ Cam ^R	[2]; this study
RN4220+MsrA	<i>Staphylococcus aureus</i> RN4220 strain transformed by pRMC2+MsrA. 10 $\mu\text{g/ml}$ Cam ^R	This study
1,4,7,8,11,14,15,16,17,18,19,20,21,23,24,25,26,27,28,30,37,38,39,40,43,54,58,62,64,70	RN4220+MsrA mutant strains with increased resistance to telithromycin. 10 $\mu\text{g/ml}$ Cam ^R	This study
24 ^C	Nutant strain 24 cured from plasmid	This study
24 ^C +pRMC2	Mutant strain 24 ^C bearing pRMC2	This study
24 ^R	Mutant strain 24 ^C transformed with pRMC2+MsrA	This study
8325-4+pRMC2	<i>Staphylococcus aureus</i> 8325-4 strain transduced with pRMC2	[3]; This study
8325-4+MsrA	<i>Staphylococcus aureus</i> 8325-4 strain transduced with pRMC2+MsrA	[3]; This study
ΔclpX +pRMC2	<i>Staphylococcus aureus</i> 8325-4 strain ΔclpX transduced with pRMC2	[3]; This study
ΔclpX +MsrA	<i>Staphylococcus aureus</i> 8325-4 strain ΔclpX transduced with pRMC2+MsrA	[3]; This study
ΔclpC +pRMC2	<i>Staphylococcus aureus</i> 8325-4 strain ΔclpC transduced with pRMC2	[4]; This study
ΔclpC +MsrA	<i>Staphylococcus aureus</i> 8325-4 strain ΔclpC transduced with pRMC2+MsrA	[4]; This study
ΔclpP +pRMC2	<i>Staphylococcus aureus</i> 8325-4 strain ΔclpP transduced with pRMC2	[3]; This study
ΔclpP +MsrA	<i>Staphylococcus aureus</i> 8325-4 strain ΔclpP transduced with pRMC2+MsrA	[3]; This study
Primers	Sequence	Description
MrsA_forward	TAAGCTCTCTATGATGGTACCTAAGGAGGCAAA TATGGAACAATATACAATTAATTTAACCAAAT CAATCATAAATTG	<i>msr(A)</i> amplification, addition of Shine-Dalgarno sequence and KpnI restriction site
MrsA_reverse	ACGGCCAGTGAATTCGAGCTCTTAATGATGATG ATGATGATGTGAACCACCTGGAGTTATATC ATGAATAGATTGCTCTGTTAATTCCC	<i>msr(A)</i> amplification, with addition of sequence coding for Gly-(His) ₆ tag and SacI restriction site
MsrA_MUT_F	TTATTTGGATCCCCCTCGAGTT	<i>msr(A)</i> sequencing
MsrA_MUT_R	TGTGCTGCAAGGCGATTA	<i>msr(A)</i> sequencing
ClpX_Forward	TTTTGGTACCTGTTGCATTGTAACATCCAATCTA GTATAGTC	<i>clpX</i> amplification
ClpX_Reverse	TTTTGGATCCTAATGATTAATTCTATATTATTAG GATTAACCTTTTCATTTTATATCCTC	<i>clpX</i> amplification
ClpX_Seq_1	GGCACACGTCCGATAAATTC	<i>clpX</i> sequencing
ClpX_Seq_2	TGACGTTTCAGGTGAAGGTG	<i>clpX</i> sequencing

References

1. Corrigan RM, Foster TJ (2009) An improved tetracycline-inducible expression vector for *Staphylococcus aureus*. *Plasmid* **61**: 126–129.
2. Nair D, Memmi G, Hernandez D, Bard J, Beaume M, Gill S, Francois P, Cheung AL (2011) Whole-genome sequencing of *Staphylococcus aureus* strain RN4220, a key laboratory strain used in virulence research, identifies mutations that affect not only virulence factors but also the fitness of the strain. *J Bacteriol* **193**: 2332–2335.
3. Frees D, Qazi SN a., Hill PJ, Ingmer H (2003) Alternative roles of ClpX and ClpP in *Staphylococcus aureus* stress tolerance and virulence. *Mol Microbiol* **48**: 1565–1578.
4. Frees D, Chastanet A, Qazi S, Sørensen K, Hill P, Msadek T, Ingmer H (2004) Clp ATPases are required for stress tolerance, intracellular replication and biofilm formation in *Staphylococcus aureus*. *Mol Microbiol* **54**: 1445–1462.